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Halothane Hepatotoxicity and the Reduced Derivative, 1,1,1-Trifluoro-2-chloroethane

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Halothane (1,1,1-trifluoro-2-bromo-2-chloroethane) is a safe, clinically useful inhalation anesthetic. Rare, unpredictable cases of liver necrosis have been reported following its use. Although the mechanism of this reaction in man is unknown the most plausible is biotransformation to reactive intermediates compounds. The oxidative metabolism of halothane appears to be benign. There is early evidence that reductive (nonoxygen dependent) may be harmful. Since the bromine atom of halothane appears to possess weak bond energy, the reduced, debrominated derivative of halothane, 1,1,1-trifluoro-2-chloroethane, was synthesized and tested for hepatotoxicity in the rat. The derivative is unstable and thus was prepared anaerobically and trapped in propylene glycol solvent. Injection of small amounts of this compound into the portal vein of rats produces extensive liver necrosis. It is postulated that biotransformation of halothane via a reductive pathway could produce this reactive intermediate metabolite.

Introduction

The volatile anesthetic halothane (1,1,1-trifluoro-2-bromo-chloroethane) was introduced into clinical practice in the U.S. in 1958 and has subsequently become the most commonly employed drug of this category. There are many reasons to endorse this popularity. It is easy to use, potent, nonflammable, and possesses little in the way of post-operative nausea or vomiting propensities. The low blood/air partition coefficient (2.1) of the anesthetic endows it with rapid induction characteristics.

Soon after initial widespread clinical use, reports appeared implicating halothane in the development of post-anesthetic hepatic necrosis (*1, 2*). Although such anecdotal case reports strongly implicated a causal relationship, the rarity and unpredictability of the syndrome was underlined by several large prospective and retrospective studies which were inconclusive. (*3–5*). Due to lack of animal models of halothane toxicity, a nonstatistical inference that second administrations of the anesthetic increased the incidence of hepatitis, and positive "challenge tests" in susceptible individuals, it became vogue to attribute the hepatic destruction to a peculiar allergy or hypersensitivity limited to the human species (*6–8*). It must be pointed out however that a positive drug "challenge" only indicates that an individual is capable of an idiosyncratic reaction and does not pinpoint mechanism of that reaction. In addition the lymphocyte stimulation test, the only concrete evidence upon which halothane hypersensitivity theory was based has been disproven by another group (*9*).

It was Van Dyke's and Stier's groups working independently that determined that halothane was extensively biotransformed by man and animals, both *in vivo* and *in vitro* (*10, 11*). The primary metabolic products formed were trifluoroacetic acid and bromide. The mechanism is by an oxidative reaction employing the classic NADPH-O_2 dependent mixed function oxidase system. This oxidative pathway is apparently innocuous and does not produce hepatic damage even in the presence of phenobarbital induction. Later work has indicated that nonoxygen-dependent ("reductive") biotransformation may produce metabolites with sufficient

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reactivity to initiate hepatic necrosis. It is the purpose of this article to investigate this possibility.

Methods

Since the bonding energy of the bromine atom in halothane is relatively weak, it is theoretically possible that an enzymatic reductive attack could occur at this site. Therefore the reduced, debrominated produce of halothane, 1,1,1-trifluoro-2-chloroethane, was synthesized and studied for hepatotoxic potential.

Synthesis of 1,1,1-Trifluoro-2-chloroethane

The general reaction employed was:

trifluoroethanol (TFE) + $PCl_3 \rightarrow$

1,1,1-trifluoro-2-chloroethane + HCl

An airtight glassware apparatus was thoroughly cleansed and dried overnight in an oven. The system was set up and flushed for 30 min with pure N_2. An anaerobic sidearm buret containing PCl_3 was attached to the reaction flask which contained 5 ml pure TFE. Then 10 ml saturated PCl_3 was slowly added to the TFE at ambient temperature and stirred. The reaction was considered complete when bubbling ceased. Two traps surrounded by a Dry Ice/acetone mixture were placed distal to the reaction flask. The first trap was to eliminate any water condensate, and the second trap contained anhydrous propylene glycol, 5 ml. By use of two stopcocks, the effluent 1,1,1-trifluoro-2-chloroethane was directed and dissolved into the propylene glycol. This product was stable in this solvent as long as it was stored in Dry Ice. Yield of this reaction was approximately 1 g. Purity was greater than 99% determined by hydrolysis and chloride gravimetry and by infrared spectroscopy. HCl was eliminated by absorption and condensation.

Hepatotoxicity of 1,1,1-Trifluoro-2-chloroethane

This compound is extremely reactive, with a tendency to explode on contact with water. Previous data in man indicate that approximately 18% of absorbed halothane is biotransformed in man (*10*). Extrapolation of these data to 250 g male Sprague-Dawley rats means that approximately 6.5 mg of halothane would be metabolized in this species primarily, it was assumed, by the oxidative route. Therefore, ca. 1 mg of 1,1,1-trifluoro-1-chloroethane in 0.5 ml propylene glycol was directly injected into the portal vein of rats under pentobarbital anesthesia (25 mg/kg IP). After suturing the abdominal cavity the animals were placed in a cage with water and food *ad libitum* and sacrificed 24 hr later. After sacrifice, liver slices were taken, fixed, stained with hematoxylin and eosin, and examined. Propylene glycol (0.5 ml) was used as control.

Results

Figure 1 illustrates the extensive coagulation necrosis produced by injection of the 1,1,1-trifluoro-2-chloroethane. All animals administered this compound displayed this pattern. Propylene glycol alone was innocuous.

Discussion

There is recent evidence from several groups of investigators when placed in composite, indicating that nonoxygen-dependent biotransformation of halothane may be a potential vector of hepatotoxicity. Uehleke et al. (*12*) first indicated that covalent binding of halothane metabolites to phenobarbital pretreated rabbit hepatic microsomal protein was enhanced if incubated in an anaerobic atmosphere. It was speculated by this group that this irreversible binding to endoplasmic reticulum proteins could be connected with halothane hepatotoxic effects. Wood et al. (*13*) also confirmed enhanced covalent binding of halothane metabolites in an anaerobic atmosphere, but found such binding higher in microsomal phospholipids than in microsomal protein. In this last study, ^{36}Cl-halothane metabolites had similar binding characteristics to ^{14}C-halothane, strongly implying dechlorination did not play a role in activation to reactive intermediates. Cohen's group reported that halothane was defluoridated to small amounts of difluorobromo-chloroethane mercapturic acid in man (*14*). Following this disclosure in man, Widger et al. (*15*) found that there is enhanced defluoridation of halothane in phenobarbital induced rats anesthetized in a low (7%) oxygen atmosphere. Associated with this metabolic alteration, these investigators found centrolobular necrosis of the liver.

In vivo evidence for the hepatotoxic potential of induced halothane metabolism in the presence of low oxygen tension has been confirmed by McLain et al. (*16*). Anesthesia with 1% halothane in 14% oxygen (85% N_2) for 2 hr produces centrilobular necrosis and rises in plasma SGOT and SGOT within 24 hr in the rat. No evidence of hepatotoxicity is seen in phenobarbital pretreated control animals subjected to 85% N_2 for a similar duration. In addition, pretreatment of rats with high dosages of the potent reductive enzyme inducing compound Aroclor 1254 (a polychlorinated biphenyl) produces

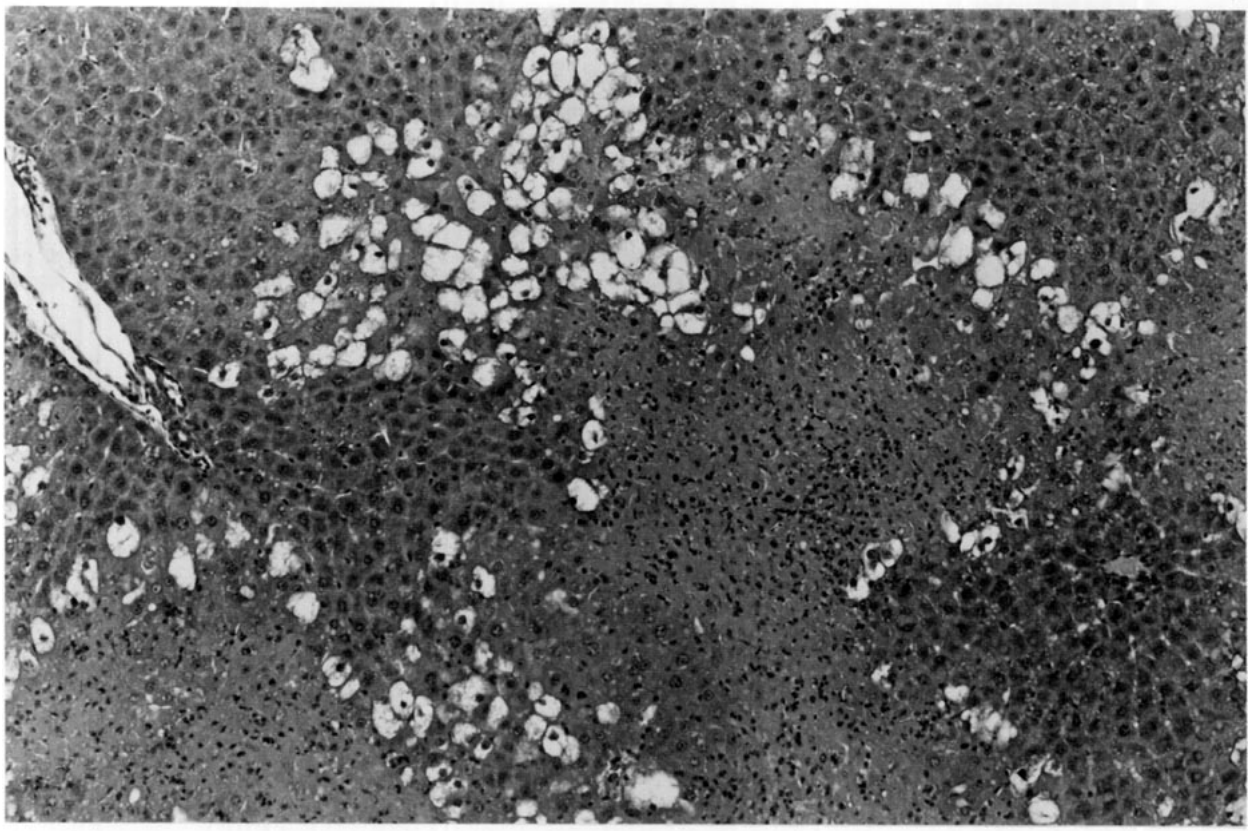

FIGURE 1. Extensive necrosis of the liver 24 hr following injection of approximately 2 mg 1,1,1-trifluoro-2-chloroethane in the portal vein of a 250 g Sprague-Dawley rat.

centrilobular necrosis following halothane anesthesia, even in the presence of 99% oxygen (*17*). In no *in vivo* studies has the possibility of an early formation of 1,1,1-trifluoro-2-chloroethane been critically investigated as yet.

Although the exact offending metabolite is unknown, it is highly likely that "reductive" (nonoxygen-dependent) biotransformation of halothane can produce reactive intermediates of sufficient reactivity to destroy liver cells. Reductive debromination to 1,1,1-trifluoro-2-chloroethane is a possible metabolite and has been found in this study to be capable of destruction of liver morphology.

Summary

There is likelihood that reductive (nonoxygen-dependent) biotransformation of halothane may lead to reactive intermediates capable of producing hepatotoxicity. One such intermediate which may theoretically be formed by such a pathway is the reduced, debrominated derivative of halothane, 1,1,1-trifluoro-2-chloroethane. This compound has been synthesized and found to be quite reactive. When appropriate amounts of this product are injected into the portal veins of rats, extensive necrosis of the liver results.

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